

**PRELIMINARY AMENDMENT
CONT of USSN 09/700,397**

AMENDMENTS TO THE SPECIFICATION

Please replace the present title with the following rewritten title:

NOVEL POLYPEPTIDES, CDNA ENCODING THE SAME AND UTILIZATION
THEREOF

Page 2, after line 7, please amend the specification by inserting the sentence:

This is a Continuation of Application No. 09/700,397, filed November 14, 2000;
which is a 371 of PCT/JP99/02485, filed May 13, 1999, the disclosure of each of which is
incorporated herein by reference.

Page 5, lines 12-30 and Page 6, line 1, are amended as follows:

[The structure of the Invention]

The present invention relates to

- (1) a polypeptide comprising an amino acid sequence of SEQ ID NOS. ~~13~~, 4, ~~6, 9, 11~~ or ~~12~~14,
- (2) a cDNA encoding the polypeptide described in (1),
- (3) a cDNA comprising a nucleotide sequence of SEQ ID NOS. ~~2, 5, 7, 10~~1, 5, 6, 9 or ~~13~~12,
and
- (4) a cDNA comprising a nucleotide sequence of SEQ ID NOS. ~~3, 8, 11~~2, 7, 10 or ~~14~~13.

The present invention relates to a substantially purified form of the polypeptide
comprising the amino acid sequence shown in SEQ ID NOS. ~~1, 4, 6, 9~~ or ~~12~~3, 4, 8, 11 or 14,
homologue thereof, fragment thereof or homologue of the fragment.

Further, the present invention relates to cDNAs encoding the above peptides. More
particularly the invention is provided cDNAs comprising nucleotide sequence shown in SEQ
ID NOS. ~~2, 5, 7, 10~~ or ~~13~~1, 5, 6, 9 or 12, and cDNA containing a fragment which is
selectively hybridizing to the cDNA comprising nucleotide sequence shown in SEQ ID NOS.

PRELIMINARY AMENDMENT
CONT of USSN 09/700,397

~~2, 5, 7, 10, 13, 3, 8, 11 or 14~~1, 5, 6, 9, 12, 2, 7, 10 or 13. A said cDNA capable for hybridizing to the cDNA includes the contemporary sequence of the above sequence.

Page 6, lines 2-28, are amended as follows:

A polypeptide comprising amino acid sequence shown in SEQ ID NOS. ~~1, 4, 6, 9 or 123, 4, 8, 11 or 14~~ in substantially purified form will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is that of the SEQ ID NOS. ~~1, 4, 6, 9 or 123, 4, 8, 11 or 14~~.

A homologue of polypeptide comprising amino acid sequence shown in SEQ ID NOS. ~~1, 4, 6, 9 or 123, 4, 8, 11 or 14~~ will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the polypeptide comprising the said amino acid sequence over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 more contiguous amino acids. Such a polypeptide homologue will be referred to a polypeptide of the present invention.

Further, a fragment of polypeptide comprising amino acid sequence shown in SEQ ID NOS. ~~1, 4, 6, 9 or 123, 4, 8, 11 or 14~~ or its homologues will be at least 10, preferably at least 15, for example 20, 25, 30, 40, 50 or 60 amino acids in length.

A cDNA capable of selectively hybridizing to the cDNA comprising nucleotide sequence shown in SEQ ID NOS. ~~2, 5, 7, 10, 13, 3, 8, 11 or 14~~1, 5, 6, 9, 12, 2, 7, 10 or 13 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the cDNA comprising the said nucleotide sequence over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 or more contiguous nucleotides. Such a cDNA will be referred to "a cDNA of the present invention".

PRELIMINARY AMENDMENT
CONT of USSN 09/700,397

Fragments of the cDNA comprising nucleotide sequence shown in SEQ ID NOS. ~~2, 5, 7, 10, 13, 3, 8, 11 or 14~~ 1, 5, 6, 9, 12, 2, 7, 10 or 13 will be at least 10, preferably at least 15, for example 20, 25, 30 or 40 nucleotides in length, and will be also referred to "a cDNA of the present invention" as used herein.

Page 7, lines 6-11, are amended as follows:

A further embodiment of the present invention provides host cells transformed with the vectors for the replication and expression of the cDNA of the present invention, including the cDNA comprising nucleotide sequence shown in SEQ ID NOS. ~~2, 5, 7, 10, 13, 3, 8, 11 or 14~~ 1, 5, 6, 9, 12, 2, 7, 10 or 13 or the open reading frame thereof. The cells will be chosen to be compatible with the vector and may for example be bacterial, yeast, insect cells or mammalian cells.

Page 8, lines 4-27, are amended as follows:

The polypeptide of the present invention specified in (1) includes that which a part of their amino acid sequence is lacking (e.g., a polypeptide comprised of the only essential sequence for revealing a biological activity in an amino acid sequence shown in SEQ ID NO. ~~13~~), that which a part of their amino acid sequence is replaced by other amino acids (e. g., those replaced by an amino acid having a similar property) and that which other amino acids are added or inserted into a part of their amino acid sequence, as well as those comprising the amino acid sequence shown in SEQ ID NOS. ~~1, 4, 6, 9 or 12~~ 3, 4, 8, 11 and 14.

As known well, there are one to six kinds of codon as that encoding one amino acid (for example, one kind of codon for Methionine (Met), and six kinds of codon for Leucine (Leu) are known). Accordingly, the nucleotide sequence of cDNA can be changed in order to encode the polypeptide having the same amino acid sequence.

PRELIMINARY AMENDMENT
CONT of USSN 09/700,397

The cDNA of the present invention, specified in (2) includes a group of every nucleotide sequence encoding polypeptides (1) shown in SEQ ID NOS. ~~1, 4, 6, 9 or 12~~ 3, 4, 8, 11 or 14. There is a probability that yield of a polypeptide is improved by changing a nucleotide sequence.

The cDNA specified in (3) is the embodiment of the cDNA shown in (2), and indicate the sequence of natural form.

The cDNA shown in (4) indicates the sequence of the cDNA specified in (3) with natural non-translational region.

cDNA carrying nucleotide sequence shown in SEQ ID NOS. ~~3, 8, 11 or 14~~ 2, 7, 10 or 13 is prepared by the following method:

Page 11, lines 21-27, are amended as follows:

Once the nucleotide sequences shown in SEQ ID NO. ~~2, 5, 7, 10 or 13~~ 1, 5, 6, 9 or 12 are determined partially or preferably fully, it is possible to obtain DNA encode mammalian protein itself, homologue or subset. cDNA library or mRNA derived from mammals was screened by PCR with any synthesized oligonucleotide primers or by hybridization with any fragment as a probe. It is possible to obtain DNA encodes other mammalian homologue protein from other mammalian cDNA or genome library.

Page 12, lines 6-11, are amended as follows:

Once the nucleotide sequences shown in SEQ ID NOS. ~~2, 5, 7, 10 or 13~~ 1, 5, 6, 9 or 12 are determined, DNAs of the invention are obtained by chemical synthesis, or by hybridization making use of nucleotide fragments which are chemically synthesized as a probe. Furthermore, DNAs of the invention are obtained in desired amount by transforming a vector that contains the DNA into a proper host, and culturing the transformant.

**PRELIMINARY AMENDMENT
CONT of USSN 09/700,397**

Page 13, lines 2-12, are amended as follows:

In the expression of the polypeptide, for example, in a mammalian cells, for example, the expression vector is prepared by inserting the cDNA encoding nucleotide shown in SEQ ID NOS. ~~3, 8, 11 or 142~~, 7, 10 or 13 into the downstream of a proper promoter (e. g., SV40 promoter, LTR promoter, metallothionein promoter etc.) in a proper vector (e. g., retrovirus vector, papilloma virus vector, vaccinia virus vector, SV40 vector, etc.). A proper mammalian cell (e. g., monkey COS-7 cell, Chinese hamster CHO cell, mouse L cell etc.) is transformed with the expression vector thus obtained, and then the transformant is cultured in a proper medium to express the aimed secretory protein and membrane protein of the present invention by the following method.

Page 13, lines 17-25, are amended as follows:

On the other hand, in case of membrane protein as for the present invention, the aimed polypeptide was expressed on the cell membrane. A cDNA encoding the nucleotide sequence of SEQ ID NOS. ~~2, 5, 7, 10 or 13~~, 1, 5, 6, 9 or 12 with deletion of extracellular region was inserted into the said vector, transfected into the an adequate mammalian cells to secret the aimed soluble polypeptide in the culture medium. In addition, fusion protein may be prepared by conjugating cDNA fragment encoding the said mutant with deletion of extracellular region and other polypeptide, for example, Fc portion of antibody.

Page 26, lines 9-21, are amended as follows:

Preparation of yeast SST cDNA library

Double strand cDNA was synthesized by Super Script Plasmid System for cDNA Synthesis and Plasmid Cloning (Trade name, marketed from GIBCOBRL Co.) with above poly(A)⁺RNA as template and random 9mer as primer which was containing XhoI site:

PRELIMINARY AMENDMENT
CONT of USSN 09/700,397

5'-CGATTGAATTCTAGACCTGCCTCGAGNNNNNNNNN-3' (SEQ ID NO:15)

cDNA was ligated EcoRI adapter by DNA ligation kit ver. 2 (Trade name, marketed from Takara-Shuzo Co., this kit was used in all ligating steps hereafter) and digested by XhoI. cDNAs were separated by agarose-gel electrophoresis. 300~800 bp cDNAs were isolated and were ligated to EcoRI/NotI site of pSUC2 (see US Patent No. 5, 536, 637). E. Coli DH10B strains were transformed by pSUC2 with electroporation to obtain yeast SST cDNA library.

Page 27, line 20 to Page 28, line 7, are amended as follows:

Cloning of a full-length cDNA and determination of nucleotide

A full-length cDNA was cloned using Marathon cDNA Amplification Kit (Trade name, marketed from Clontech Co.) according to 3' RACE (Rapid Amplification of cDNA End) method. I. e., poly (A)⁺RNA in human adult brain tissue 27mer primer OC001-F1:

5'-GTCCTTCAGCAAAACAGTGGATTAAA-3' (SEQ ID NO:16)

containing the deduced initiation ATG codon region based on the information of nucleotide sequence obtained by SST, was prepared. PCR was performed with the said primer and adapter primer attached in the kit. A cDNA which was amplified with clone OC001 specifically, was separated with agarose-gel electrophoresis, ligated to pT7 Blue-2 T-Vector (Trade name, marketed from Novagen Co) and transfected into E. Coli DH5 a to prepare the plasmid. Nucleotide sequences of 5'-end were determined, and the existence of nucleotide sequence OC001 SST cDNA was confirmed. Nucleotide sequence of full-length OC001 SST cDNA was determined and then sequence shown in SEQ ID NO. 3-2 was obtained. An open reading frame was determined and deduced amino acid sequence and nucleotide sequence shown in SEQ ID NOS. 1, 23, 4 and 5, respectively, were obtained.

Page 28, lines 8-25, are amended as follows:

PRELIMINARY AMENDMENT
CONT of USSN 09/700,397

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OC001 of the present invention. In addition, the polypeptide of the present invention was expected to possess the transmembrane region at C-terminal and to be GPI anchor type by hydrophobicity analysis of the obtained amino acid sequence. From these results, it was proved that polypeptide of the present invention was new membrane protein. Further, the search using BLASTX, BLASTP and FASTA revealed a significant homology between clone OC001 (region of 12th~344th amino acid in SEQ ID NO. 43) and neurotrimin [Rattus norvegicus] (region of 9th~344th amino acid of Genbank Accession U16845) and opioid-binding cell adhesion molecule [Homo sapiens] (region of 9th~345th amino acid of Genbank Accession L34774). Based on these homologies, clone OC001 and nervous cell adhesion molecule family including neurotrimin and opioid-binding cell adhesion molecule were expected to share at least some activity.

Page 28, line 29 to Page 29, line 12, is amended as follows:

A full-length cDNA was cloned by the same procedure as OC001 using Marathon cDNA Amplification Kit (Trade name, marketed from Clontech Co.) according to 3'RACE. A double-strand cDNA was prepared from RNA derived from each clone, i. e., poly(A)⁺RNA of human adult brain tissue. 27mer primer OM237-F1:

5'-CCAGAAAGCACAGCCCTGATTCTGCGT-3' (SEQ ID NO:17)

containing the deduced initiation ATG codon region based on the information of nucleotide sequence obtained by SST, was prepared. PCR was performed with the said primer and

**PRELIMINARY AMENDMENT
CONT of USSN 09/700,397**

adapter primer attached in the kit. A cDNA which was amplified with clone OM237 specifically, was recloned by the same method as OC001 to determine full nucleotide sequence and obtain the sequence shown in SEQ ID NO. 87. An open reading frame was determined and deduced amino acid sequence and nucleotide sequence shown in SEQ ID NOS. 6 and 78, respectively, were obtained.

Page 30, lines 2-20, are amended as follows:

A full-length cDNA was cloned by GENETRAPPER cDNA Positive Selection System (GIBCOBRL Co.). First, dT-primed cDNA library was prepared using plasmid pSPORT1 (GIBCOBRL Co.) as a vector from poly(A)⁺RNA of human glioblastoma cell line T98G by Super Script Plasmid System for cDNA Synthesis and Plasmid Cloning (Trade name, marketed from GIBCOBRL Co.). After preparing 27mer biotinylated primer OA004-F1:

5'-biotin-ATGCACATCTTCAAGCATGCTCAG-3' SEQ ID NO:18,
based on the information of nucleotide sequence obtained by SST, plasmid hybridized specifically with the biotinylated primer were recovered from the cDNA library according to the method of Gene Trapper Kit and then transfected into E. Coli DH10B. Colony hybridization with OA004 SST cDNA which was labeled with ³²P-dCTP, as a probe, was performed by using Random Primer DNA Labeling kit (Trade name, marketed from Takara-Shuzo Co.) according to known method to isolate the positive clone and to prepare the plasmid. Full Nucleotide sequences was determined, and then sequence shown in SEQ ID NO. 4110, which was named as OA004b, was obtained. An open reading frame was determined and deduced amino acid sequence and nucleotide sequence shown in SEQ ID NOS. 9 and 4011, respectively, were obtained.

PRELIMINARY AMENDMENT
CONT of USSN 09/700,397

Page 30, line 21 to Page 31, line 7, are amended as follows:

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OA004b of the present invention. In addition, the polypeptide of the present invention was expected to possess the transmembrane region by hydrophobisity analysis of the obtained amino acid sequence. From these results, it was proved that polypeptide of the present invention was new membrane protein. However, the search using BLASTX, BLASTP and FASTA revealed a significant homology between clone OA004b (region of 171st ~311st amino acid in SEQ ID NO. 911) and Hypothetical 52.8kD protein [Caenorhabdtis elegans] (region of 299th~453rd amino acid of Swiss Prot Accession YJ95_CAEEL), and between OA004b (region of 194th~277th amino acid in SEQ ID NO. 911) and presenilin-2 [Homo sapiens] (region of 340th~416th amino acid of Genbank Accession A56993). Based on these homologies, clone OA004b and presenilin family were expected to share at least some activity.

Page 31, line 17 to Page 32, line 4, are amended as follows:

A full-length cDNA was cloned by the same procedure as OC001 using Marathon cDNA Amplification Kit (Trade name, marketed from Clontech Co.) according to 3'RACE. A double-strand cDNA was prepaed from RNA derived from each clone, i. e., poly(A)⁺RNA of HAS303. 27mer primer OAF075-F1:

5'-CCCCGGGGACATGAGGTGGATACTGTT-3' (SEQ ID NO:19)

containing the deduced initiation ATG codon region based on the information of nucleotide sequence obtained by SST, was prepared. PCR was performed with the said primer and

**PRELIMINARY AMENDMENT
CONT of USSN 09/700,397**

adapter primer attached in the kit. A cDNA which was amplified with clone OAF075B specifically, was recloned by the same method as OC001 to determine full nucleotide sequence and obtain the sequence shown in SEQ ID NO. ~~44~~13, which was named as OAF075b. An open reading frame was determined and deduced amino acid sequence and nucleotide sequence shown in SEQ ID NOS. 12 and ~~43~~14, respectively, were obtained.

Page 32, lines 5-18, are amended as follows:

It was indicated from the results of homology search for the public database of the nucleic acid sequences by using BLASTN and FASTA, and for the public database of the amino acid sequences by using BLASTX, BLASTP and FASTA, that there was no sequence identical to the polypeptide sequence and the nucleotide sequence of OAF075b of the present invention. In addition, the polypeptides of the present invention was expected to possess no transmembrane region by hydrophobisity analysis of the obtained amino acid sequences. From these results, it was proved that polypeptide of the present invention was new secretory protein. Further, the search using BLASTX, BLASTP and FASTA revealed a significant homology between clone OAF075b (region of 1st~359th amino acid in SEQ ID NO. ~~42~~14) and preprocarboxypeptidase A2 [Homo sapiens] (region of 1st~355th amino acid of Genbank Accession U19977). Based on these homologies, clone OAF075b and preprocarboxypeptidase A2 [Homo sapiens] were expected to share at least some activity.

Pages 33-61, delete in their entirety.